

Thrombogenicity Testing in the 21st Century: *Time for Alternative Strategies for Medical Devices?*

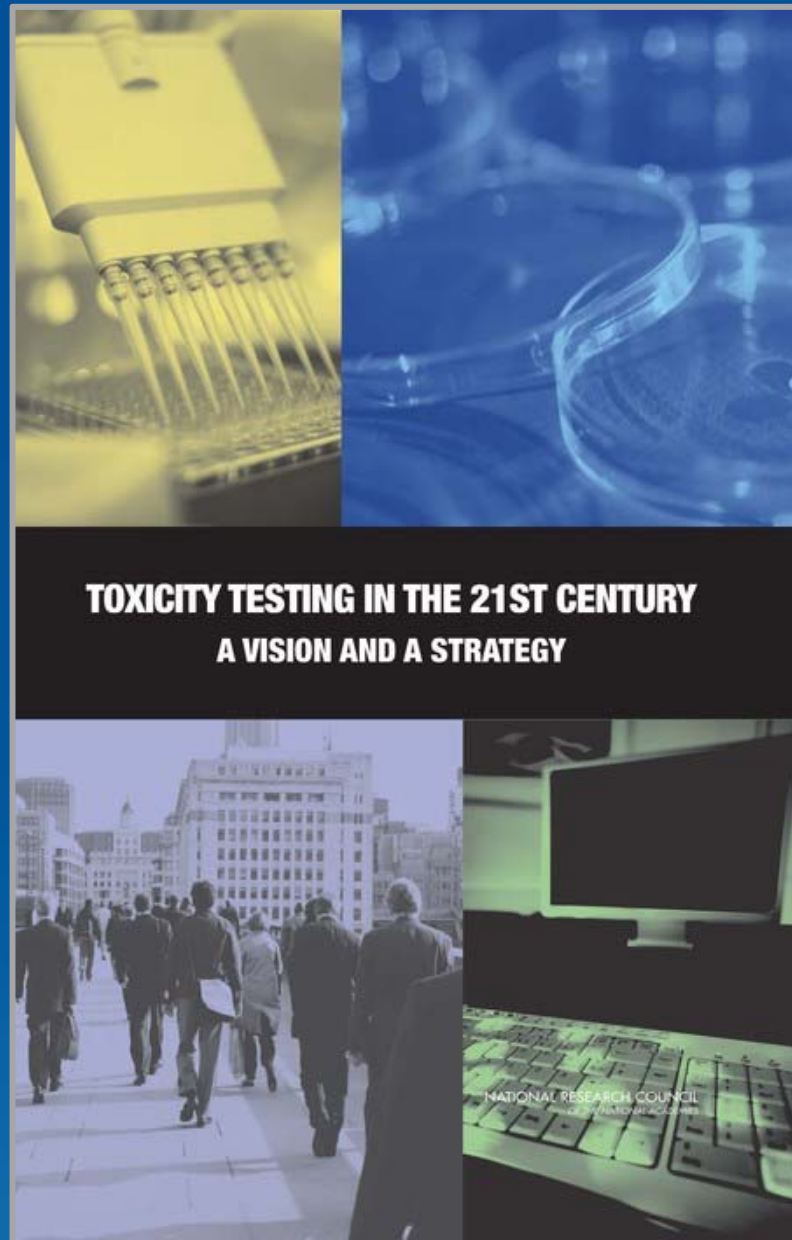
Michael F. Wolf

Scientist and Technical Fellow, Medtronic Inc.

Convenor, ISO/TC194 WG9, ISO10993 Part 4: Effects on Blood

Public Workshop:
Methods for Thrombogenicity Testing
April 14, 2014, FDA White Oak Campus

National Research Council. *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Washington, DC: The National Academies Press, 2007



BLOOD

A microscopic view of blood components, showing numerous red blood cells (erythrocytes) as biconcave discs, several white blood cells (leukocytes) with prominent nuclei, and small blue platelets (thrombocytes). The background is a warm, orange-brown color.

55% Fluid Elements

- Plasma (91% H₂O)
- 7% dissolved proteins
 - ✓ 55% albumin
 - ✓ 45% globulins
 - ✓ 7% fibrinogen, trace proteins
- 2% other stuff

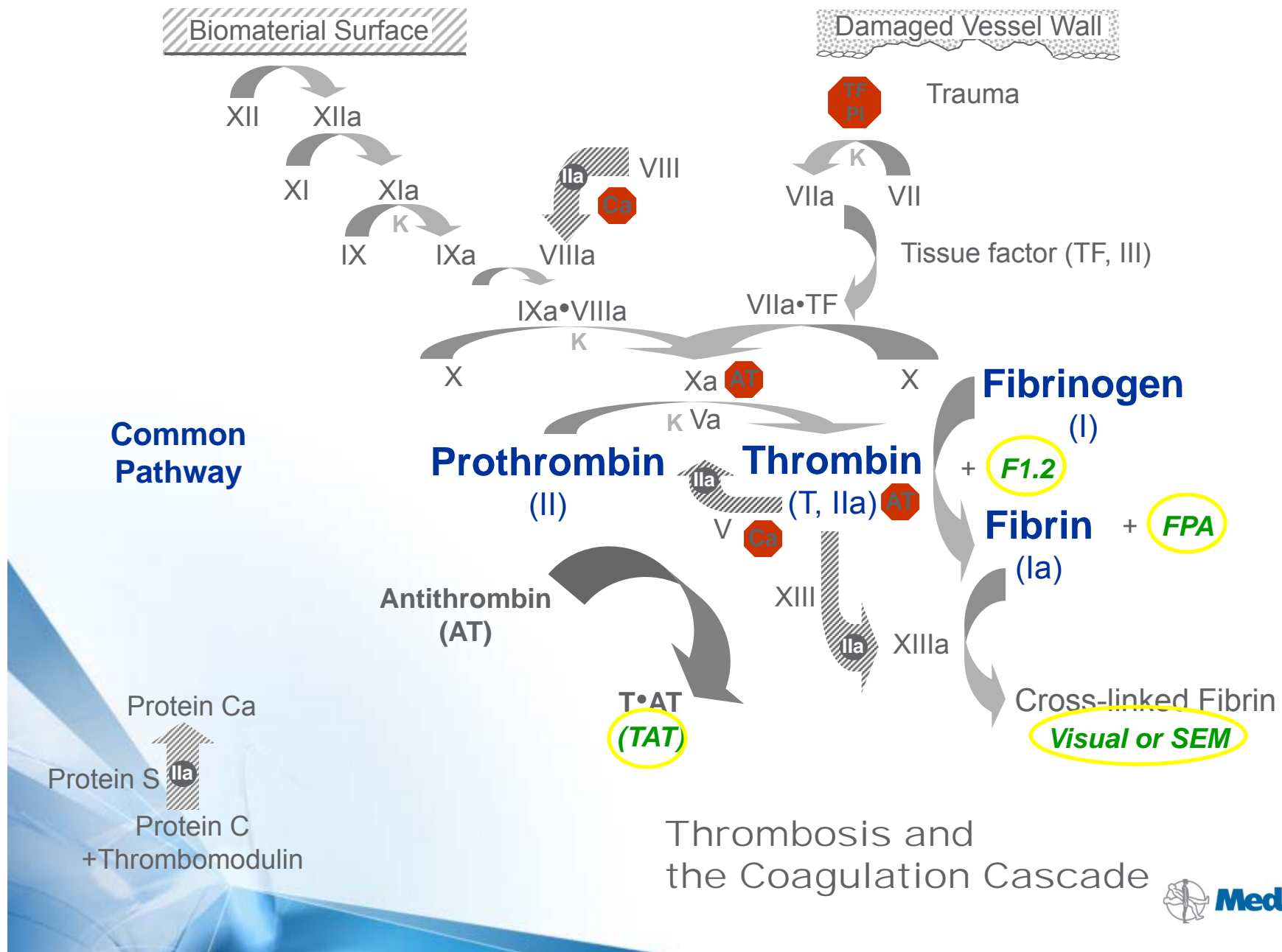
45% Formed Elements

- RBCs 5,000,000/ μ L
- Platelets 300,000/ μ L
- WBCs 7,000/ μ L

Medical device material surface

Contact Activation (intrinsic) Pathway

Tissue Factor (extrinsic) Pathway



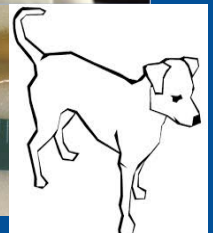
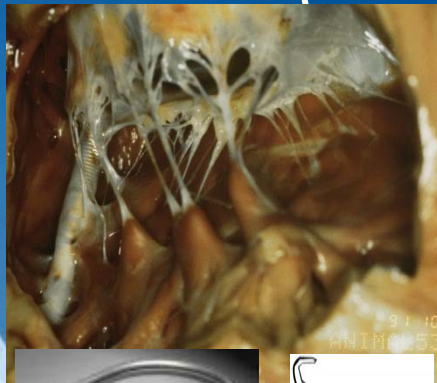
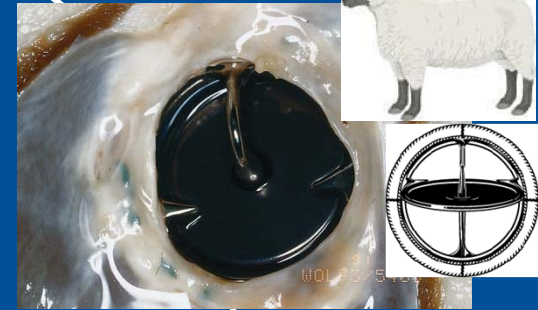
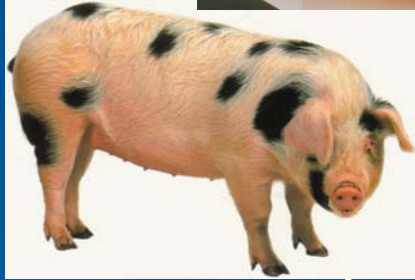
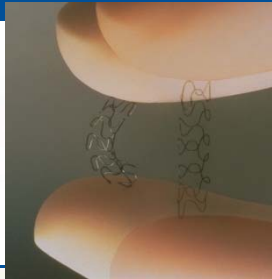
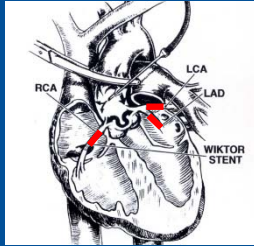
Presentation Outline

1. Background: Where are we today?
2. Background: Virchow's Triad, and Quintet
3. NAVI Model: Method; Pros and Cons
4. Example methods for medical device/material in vitro thrombogenicity testing using small-volume (3.0 mL) models of human blood

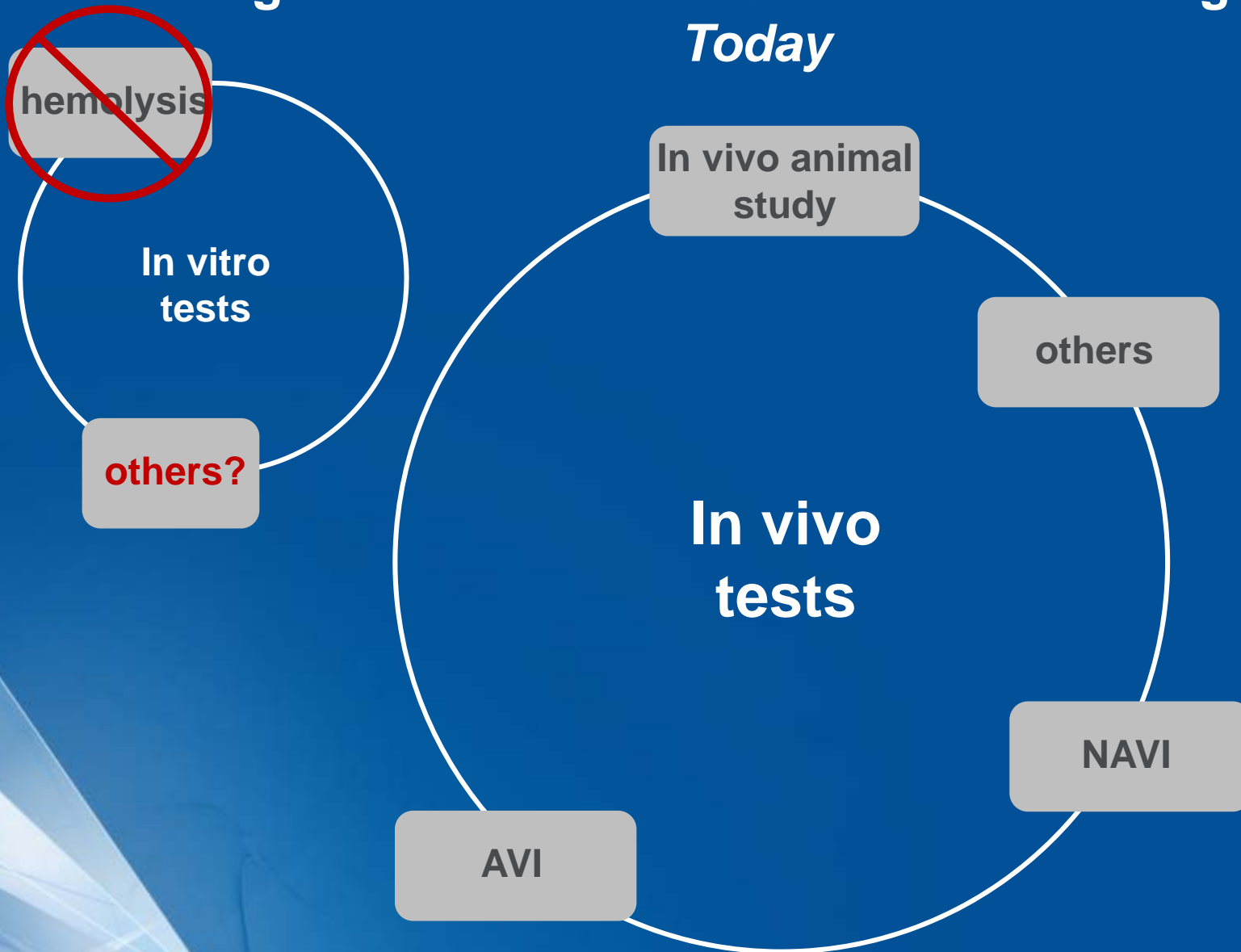
Testing for Medical Device/Material Thrombogenicity *Today*

In vivo animal
study

In vivo
tests

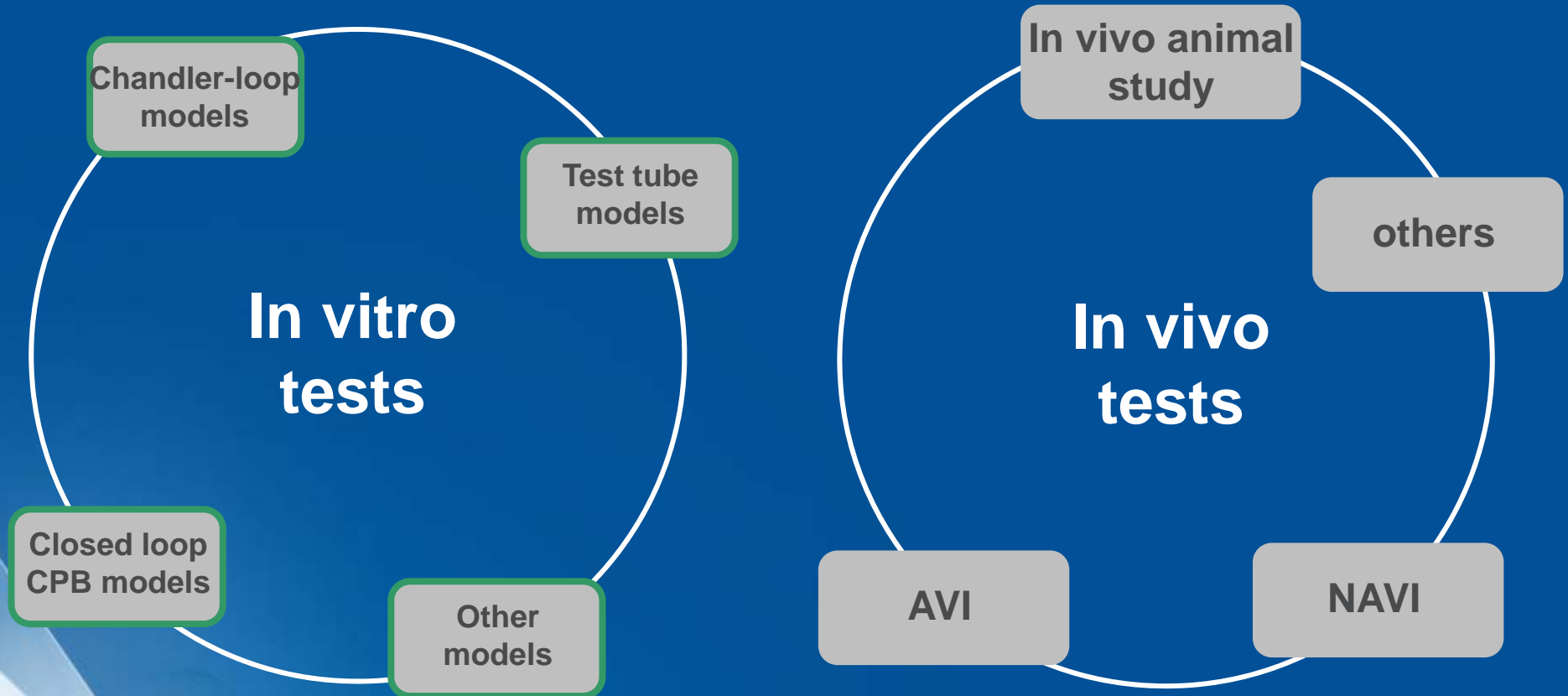


Testing for Medical Device/Material Thrombogenicity *Today*

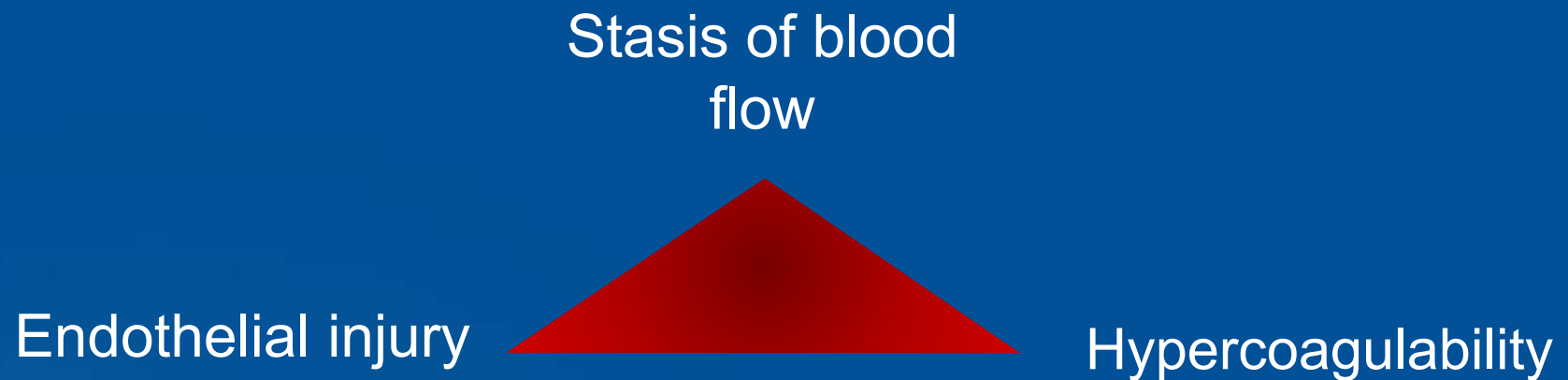


Testing for Medical Device/Material Thrombogenicity

... Time for Alternative In Vitro Strategies?

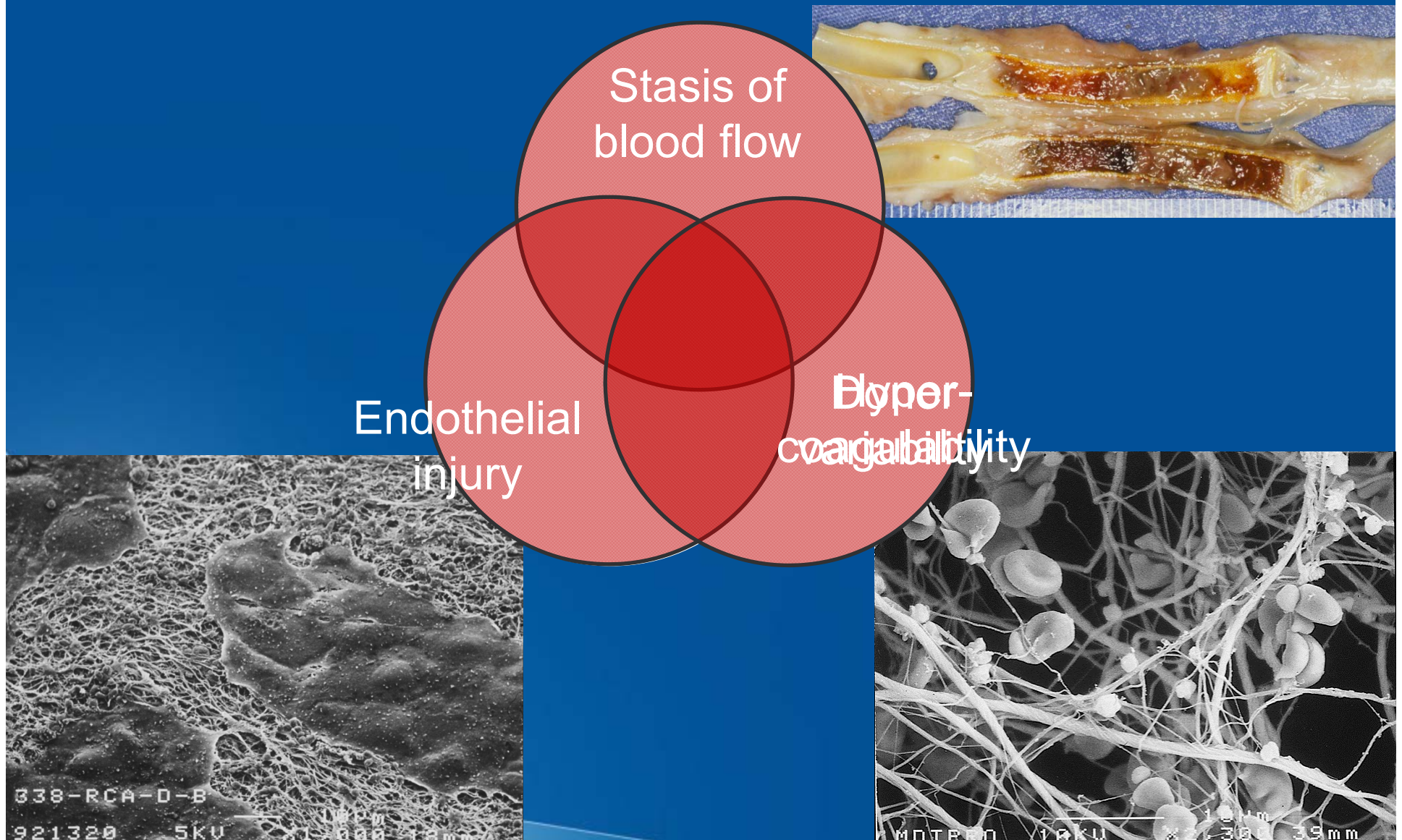


Virchow's Triad (1800s - early 1900s) *key elements of thrombosis*

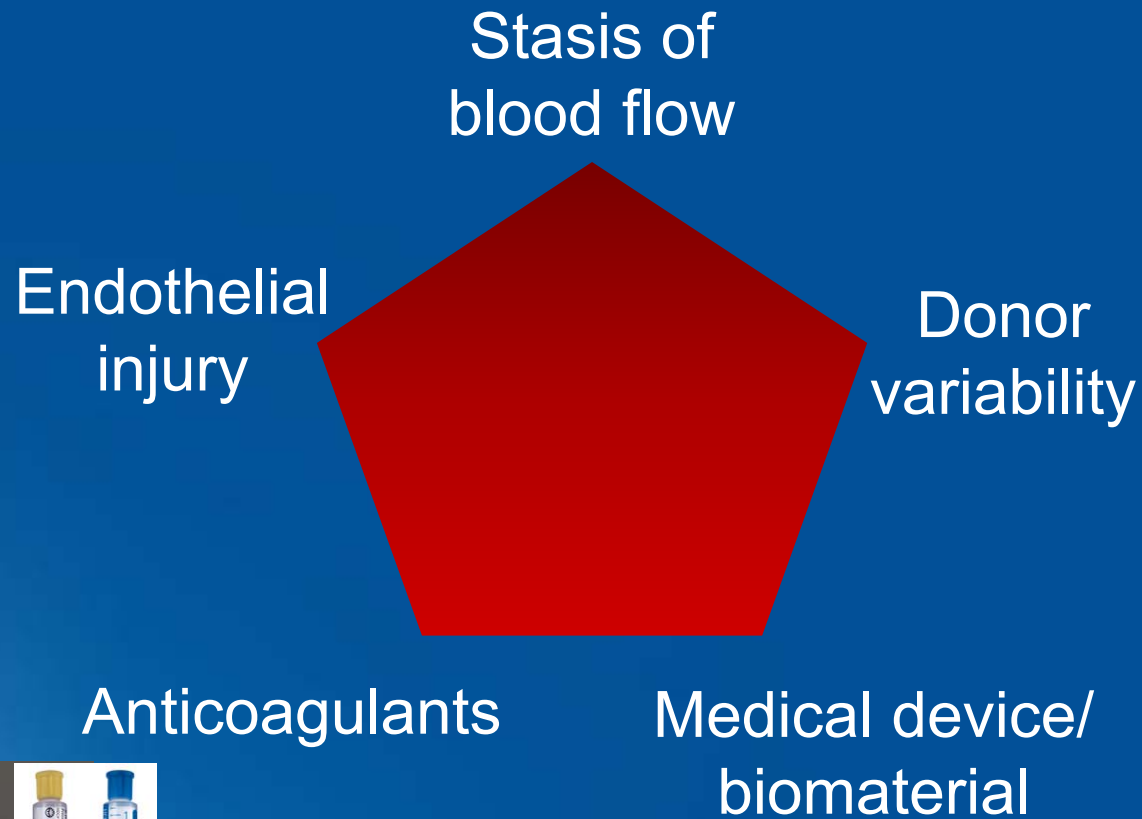


Rudolf Virchow

Virchow's Triad (Venn diagram – 21st century)

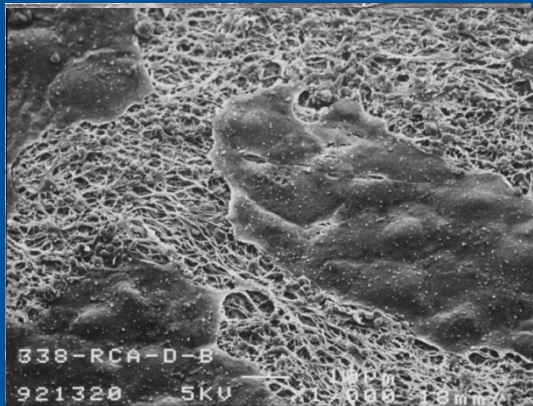
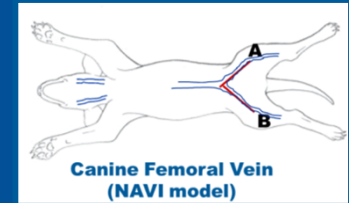


Virchow's Quintet (Pentagon)for medical devices



Biomaterial Surface

Virchow's Quintet – NAVI Model



Endothelial injury

Stasis of
blood flow



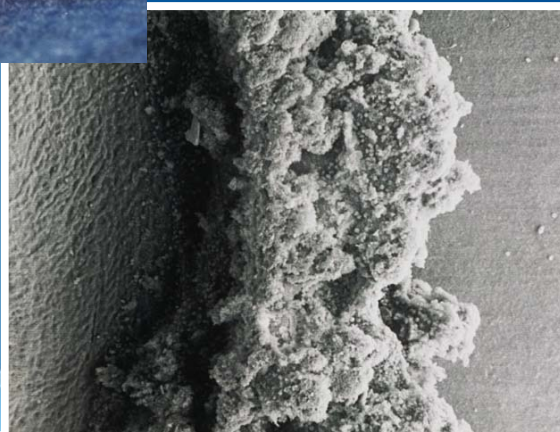
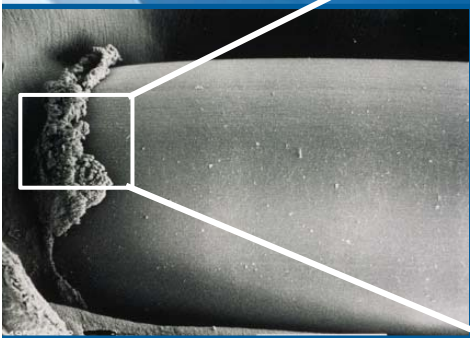
Donor variability

The effect of predetermined thrombotic potential of the recipient on small-caliber graft performance

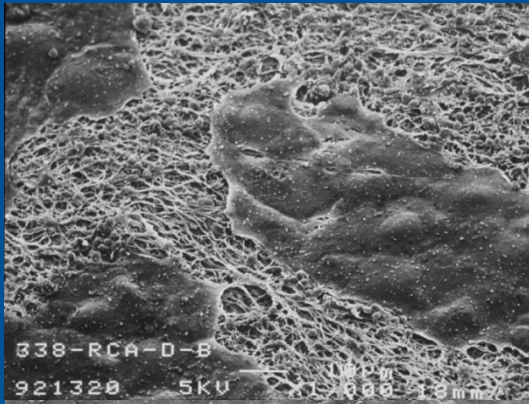
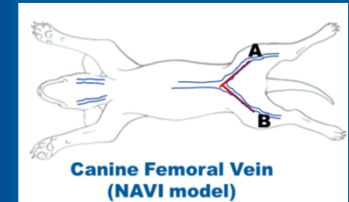
Svetlana Kaplan, Ph.D., Karen F. Marcoe, B.S., Lester R. Sauvage, M.D., Michael Zammit, M.D., Hong-De Wu, M.D., Sven R. Mathisen, M.D., and Mark W. Walker, *Seattle, Wash.*



Medical device/material



Virchow's Quintet – NAVI Model



Endothelial injury

Stasis of
blood flow



Donor variability

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Anticoagulants



Anticoagulants

Medical device/material

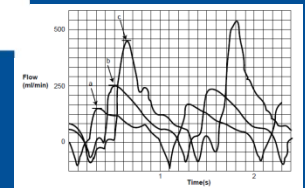
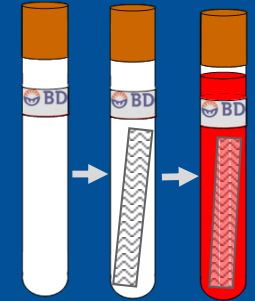
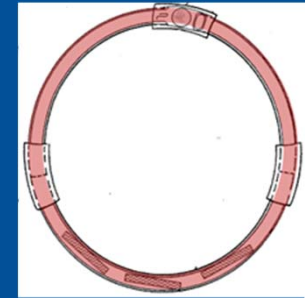


Virchow's Quintet – In vitro models



Endothelial injury

Blood flow



Donor variability

Anticoagulants Medical device/ biomaterial



Anticoagulants

Thrombin generation: phenotypic quantitation, KE Brummel-Ziedens, RL Pouliot, KG Mann, J of Thromb. and Hemost., 2, 281-288, 2003

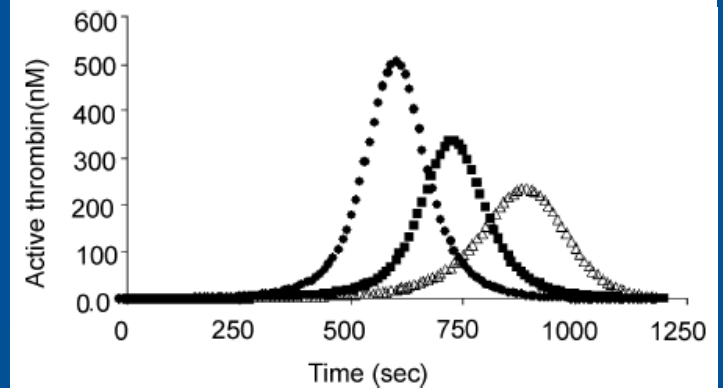


Fig. 3. Computer simulation of thrombin generation. Three individuals were modeled for active thrombin generation based upon their protein factor levels found in Table 1. Subject 3, 10 and 13 reflect the

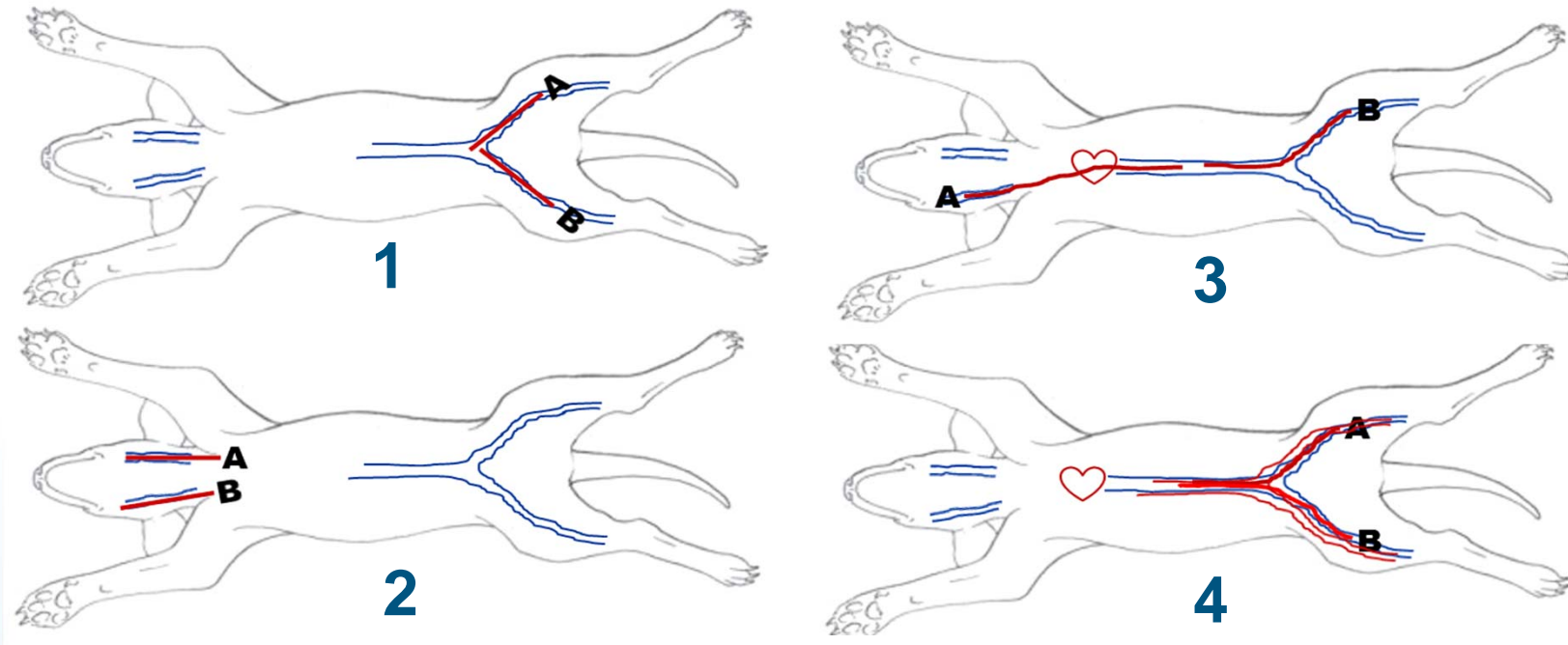
NAVI* Model: Method; Pros and Cons

*NAVI = non-anticoagulated venous implant model:



NAVI* Model: Method

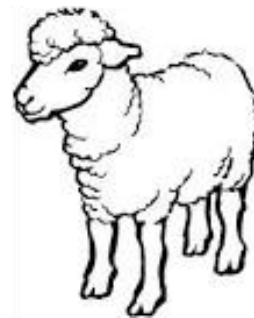
*NAVI = non-anticoagulated venous implant model:



NAVI (and AVI) model variants:



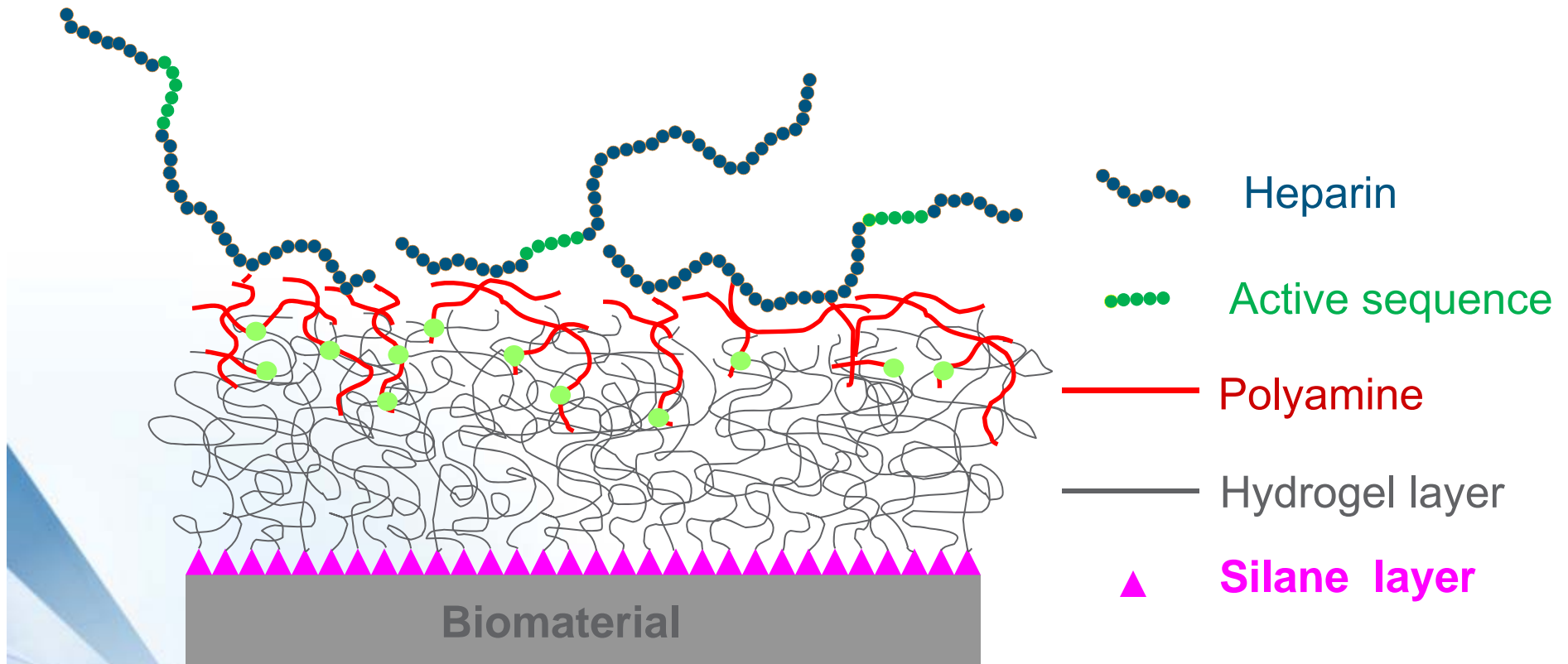
- 1 = femoral vein
- 2 = jugular vein
- 3 = IVC/SVC
- 4 = IVC-AA



NAVI* Model: Pros

*NAVI = non-anticoagulated venous implant model:

- Non-thrombogenic coating investigations



NAVI* Model: Pros

*NAVI = non-anticoagulated venous implant model:

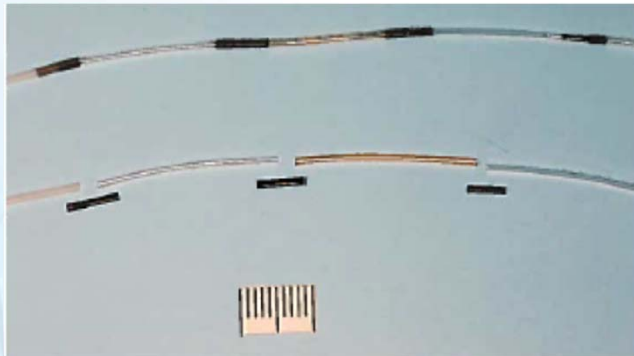
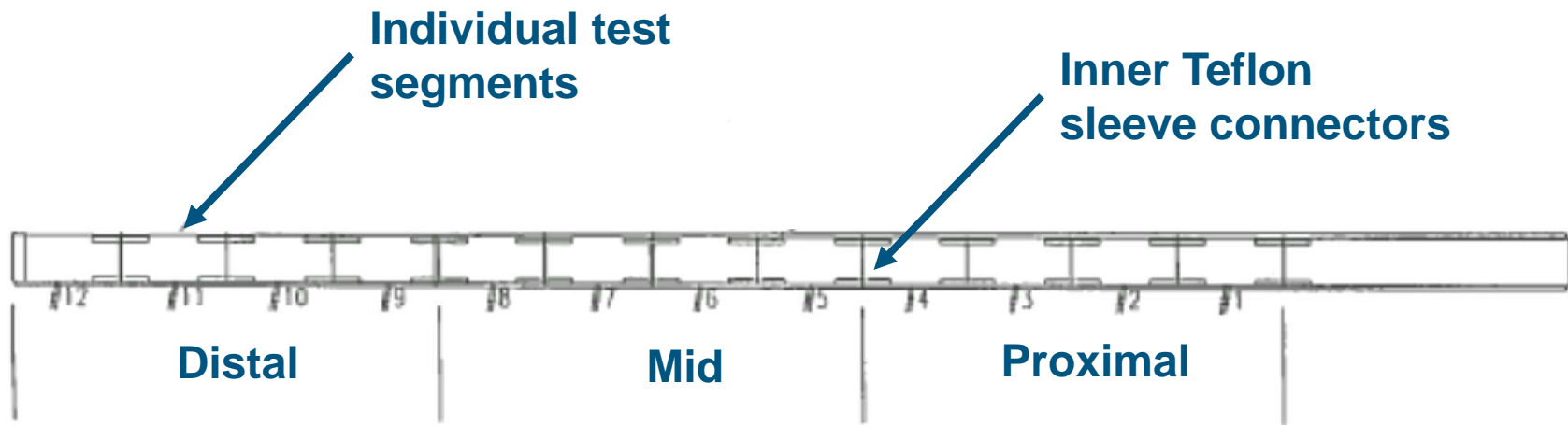
- Non-thrombogenic coating investigations



NAVI* Model: Pros

*NAVI = non-anticoagulated venous implant model:

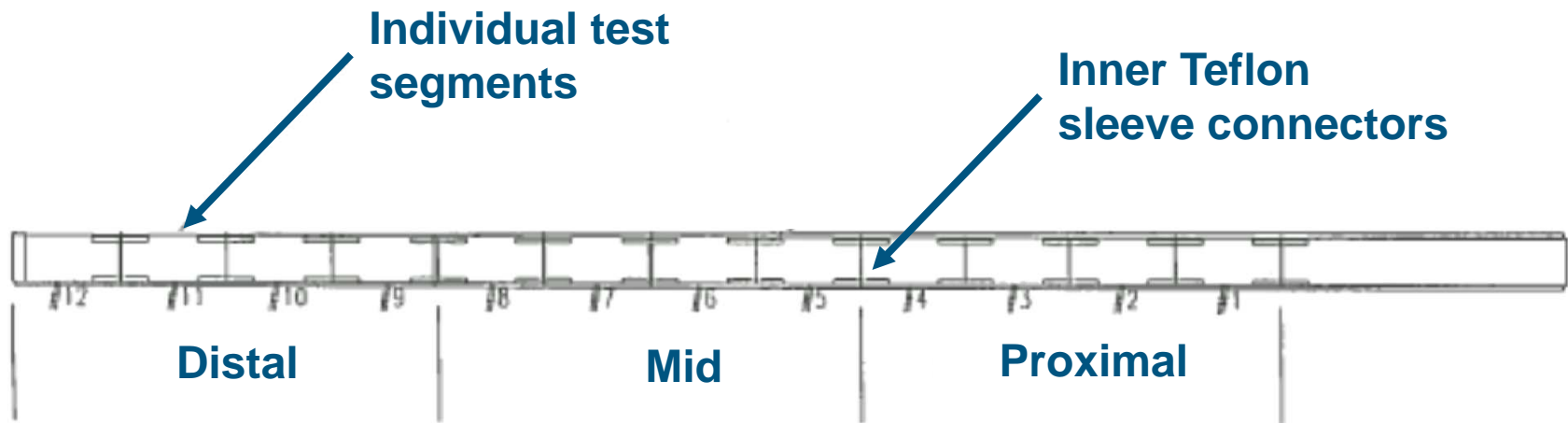
- **Non-thrombogenic coating investigations**



NAVI* Model: Pros

*NAVI = non-anticoagulated venous implant model:

- Non-thrombogenic coating investigations



NAVI* Model: Pros

*NAVI = non-anticoagulated venous implant model:

- Non-thrombogenic coating investigations
- Investigations on *thrombus formation*



NAVI* Model: Cons

*NAVI = non-anticoagulated venous implant model:

- **Scoring method variability**

Score	Thrombus Formation Score Description (typical)
0	No significant thrombosis (very small clot acceptable at insertion)
1	Minimal thrombosis, one location.
2	Minimal thrombosis, multiple locations.
3	Significant thrombosis, $\leq \frac{1}{2}$ the length of the implant, vessel patent.
4	Significant thrombosis, $> \frac{1}{2}$ the length of the implant, vessel patent.
5	Vessel completely occluded.

score ≥ 3 is considered failing / 'not meeting the requirements of the protocol'

NAVI* Model: Cons

*NAVI = non-anticoagulated venous implant model:

- Scoring method variability

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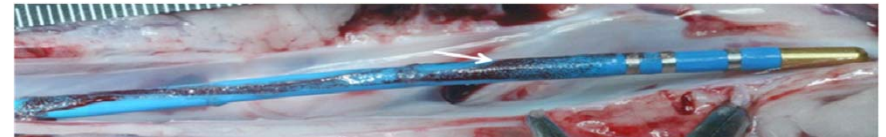
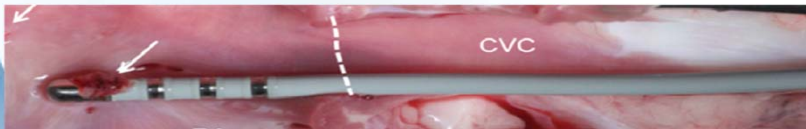


NAVI* Model: Cons

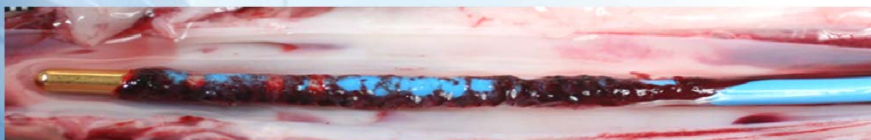
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VS.



NAVI* Model: Cons

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- **Scoring method variability**

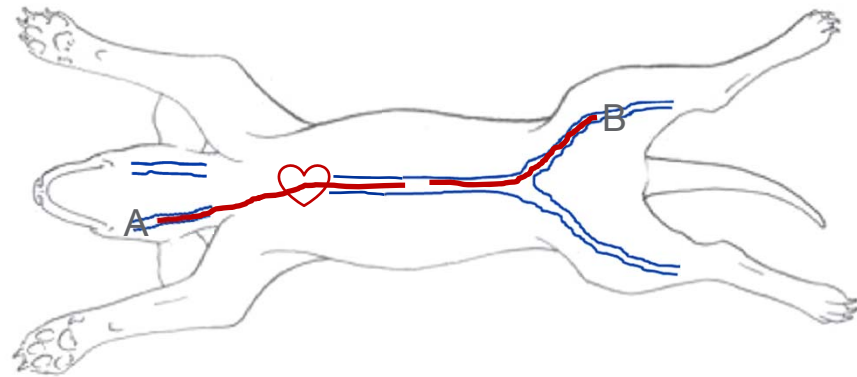
Factors that influence NAVI score*:

- ✓The implant position (P)
- ✓The implant technique (IT)
- ✓The extent of device-vessel wall contact (tissue damage, TD)
- ✓Time/incubation period (IP)
- ✓The explant technique (ET)
- ✓The material/material surface (M)
- ✓Non-thromboadherent materials get labeled non-thrombogenic (non-thromboadherent, nTA)
- ✓The recipient/subject thrombotic potential (TP)
- ✓Statistical power (SP)
- ✓Evaluator expertise (EE)

*M. F. Wolf and J. M. Anderson, Practical approach to blood compatibility assessments: general considerations and standards, in Biocompatibility and performance of medical devices, edited by Jean-Pierre Boutrand, Woodhead Publishing Ltd, (2012).

Factors that influence NAVI score:

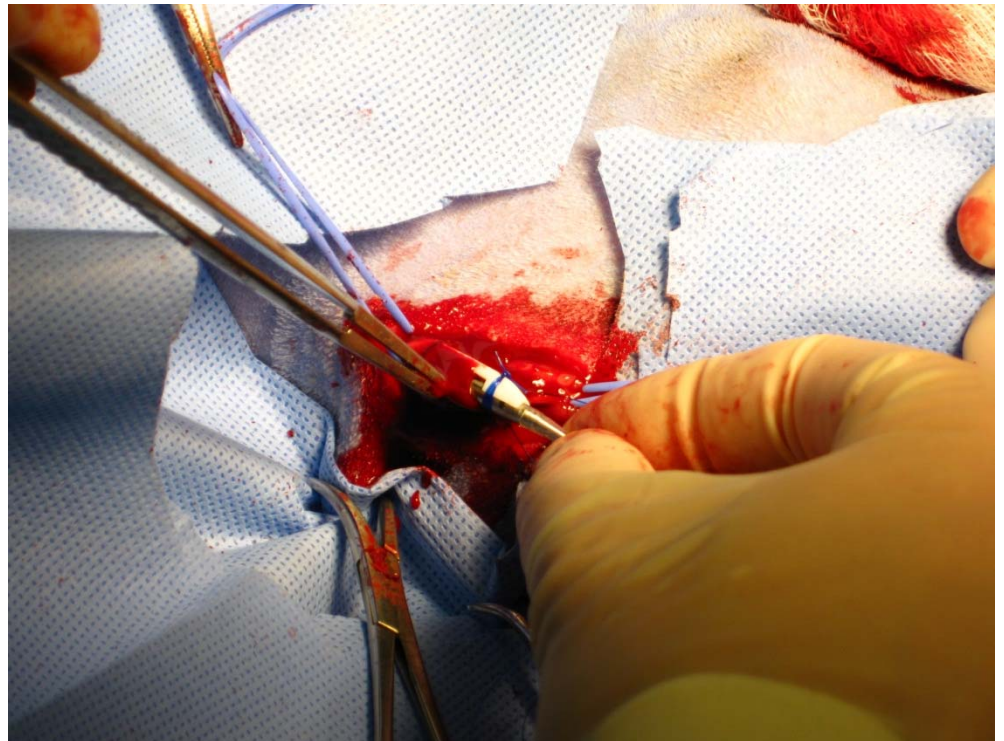
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Femoral, Jugular, IVC/SCV(?), AA(?)

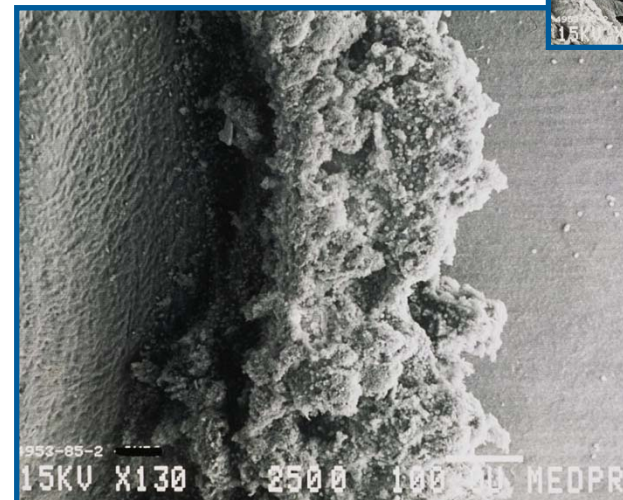
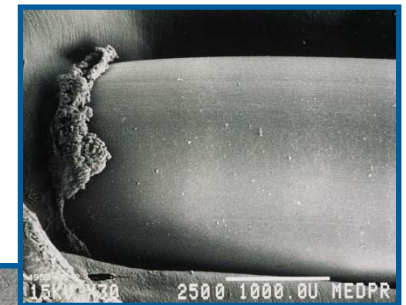
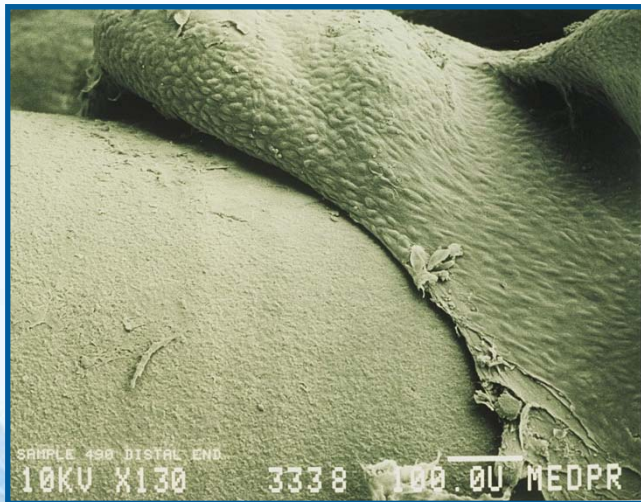
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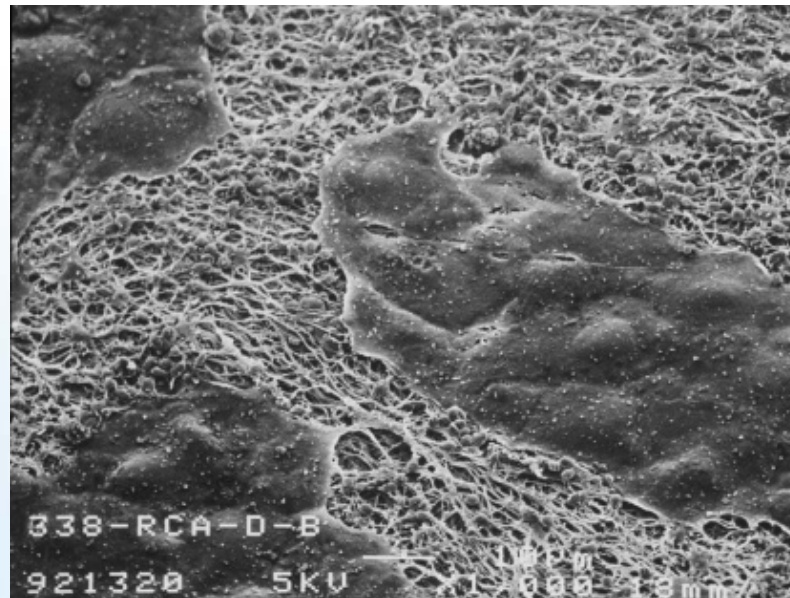
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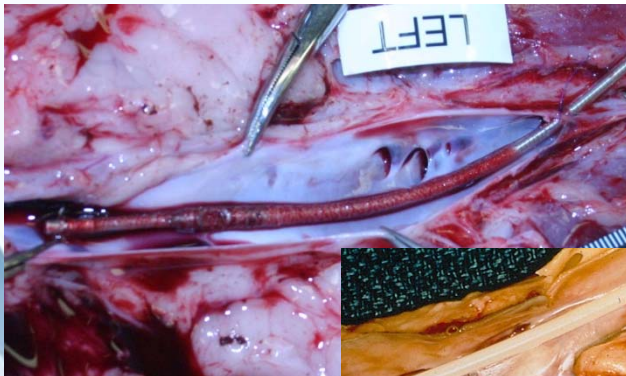
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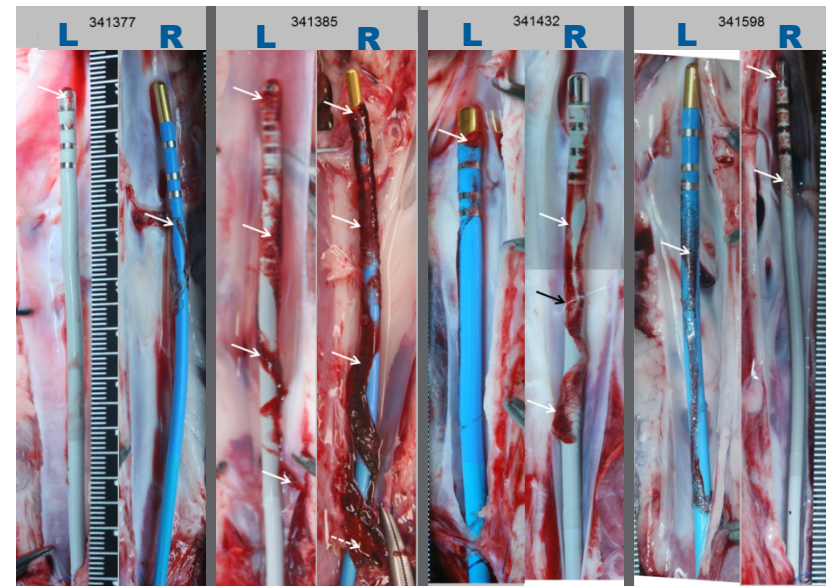


Factors that influence NAVI score:

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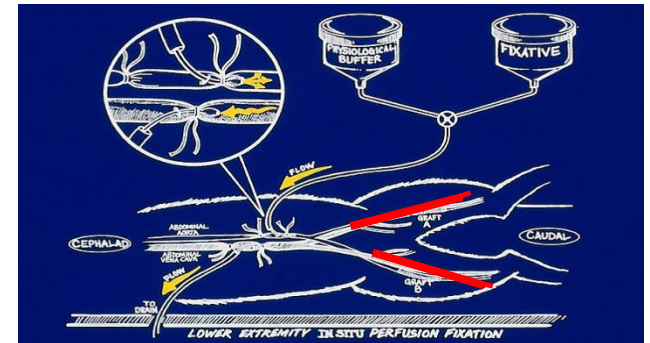
**Incubation
= 1 hour**



**Incubation
= 4 hours**

Factors that influence NAVI score:

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- ✓ Statistical power (SP)
- ✓ Evaluator expertise (EE)



Factors that influence NAVI score:

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Commercial or Near-Commercial Coatings to Improve Hemocompatibility

AOThelTM BioLASTTM

LumarTM BioGlideTM BiolineTM Blue MPC Coating

DurafloTM PyroliteTM BiolineTM Green

SPI-POLYMERTM CarmedaTM HepamedTM

PrimeGard[®] Biolite[®] Trillium[®]

PhotoLink[®] J&J Principal Materials

H-TDMAC PlasmaTFETM

Parylene MEDI-COATTM Pyrolite[®]

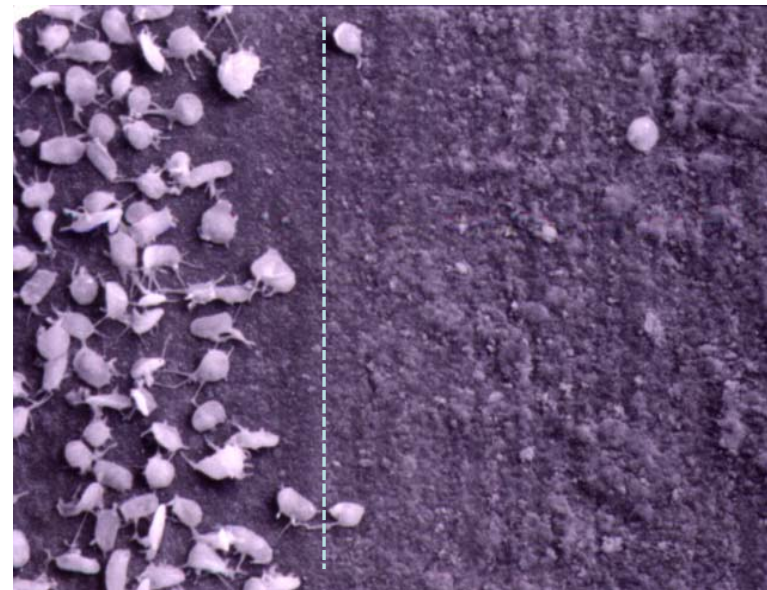
BioPhylicTM Hydak^M H-BAC

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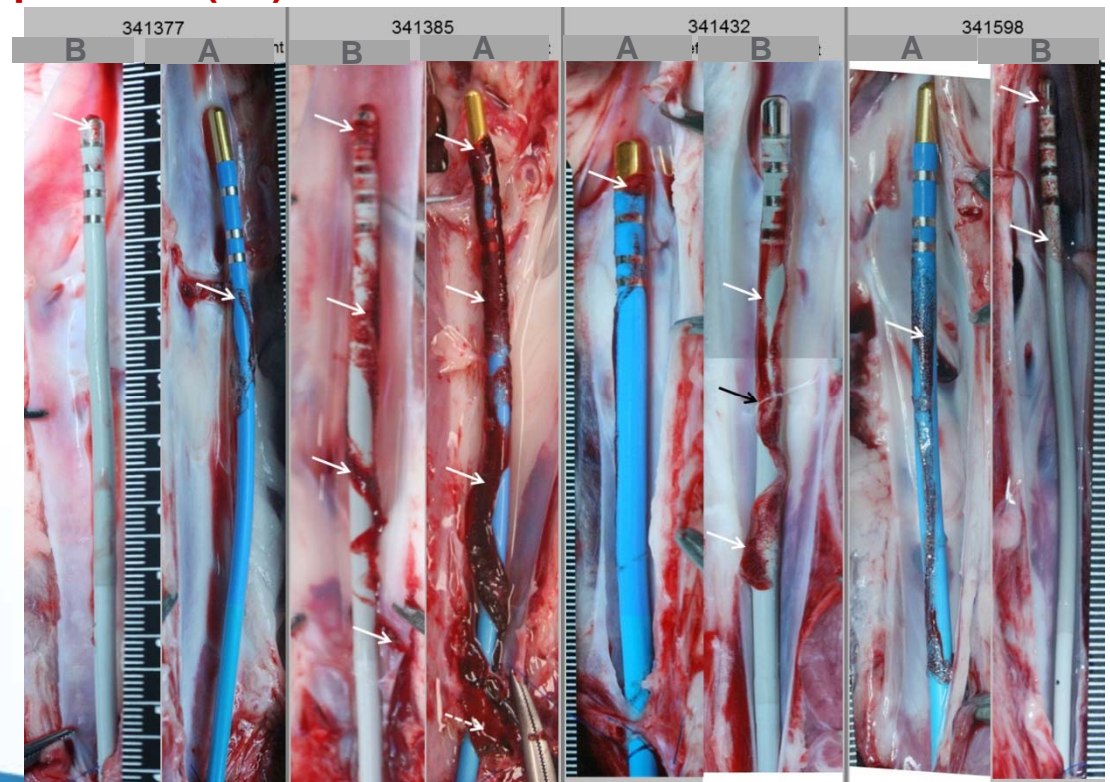


Hydrophobic Hydrophilic



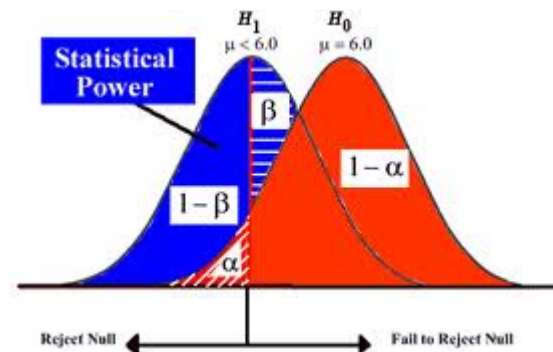
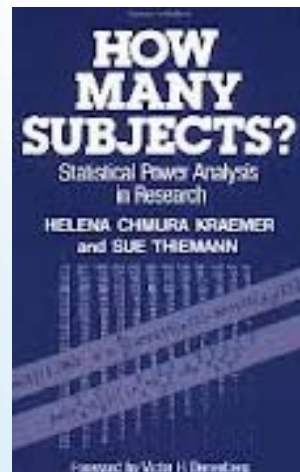
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***Biological
systems
 $n \geq 4$?***

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- ✓ Statistical power (SP)
- ✓ **Evaluator expertise (EE)**

Agonal (ante-mortem) 'clot' ≠ thrombus



NAVI* Model: Cons

*NAVI = non-anticoagulated venous implant model:

- **Scoring method variability**

Factors that influence NAVI score:

- ✓ The implant position (P)
- ✓ The implant technique (IT)
- ✓ The extent of device-vessel wall contact (tissue damage, TD)
- ✓ Time/incubation period (IP)
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Common belief:

$$\text{NAVI Score} = f(P, IT, TD, IP, ET, \mathbf{M}, nTA, TP, SP, EE)$$

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Reality:

$$\text{NAVI Score} = f(\text{P, IT, TD, IP, ET, M, nTA, TP, SP, EE})$$

AVI* Model: Cons

*AVI = anticoagulated venous implant model:

- Lack of scoring method discrimination

Factors that influence AVI score:

- ✓ Heparin concentration (H)
- ✓~~The implant position (P)~~
- ✓~~The implant technique (IT)~~
- ✓~~The extent of device-vessel wall contact (tissue damage, TD)~~
- ✓~~Time/incubation period (IP)~~
- ✓~~The explant technique (ET)~~
- ✓~~The material/material surface (M)~~
- ✓~~Non-thromboadherent materials get labeled non-thrombogenic (non-thromboadherent, nTA)~~
- ✓~~The recipient/subject thrombotic potential (TP)~~
- ✓~~Statistical power (SP)~~
- ✓~~Evaluator expertise (EE)~~

Reality:

$$\text{AVI Score} = f(H, P, IT, TD, IP, ET, M, nTA, TP, SP, EE)$$

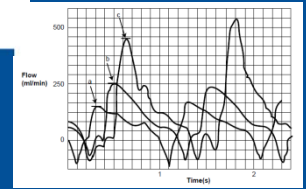
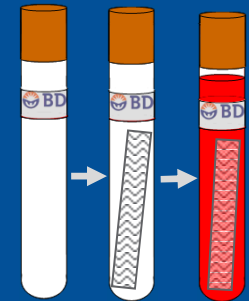
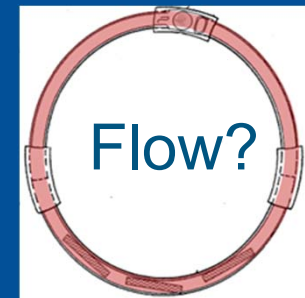
Presentation Outline

1. Background: Where are we today?
2. Background: Virchow's Triad, and Quintet
3. NAVI Model: Method; Pros and Cons
4. Example methods for medical device/material in vitro thrombogenicity testing using small-volume (3.0 mL) models of human blood

Examples methods:



Stasis of blood flow



Impact of time between draw and use



K. Münch, M. F. Wolf, E. J. Fogt, P. Schroeder, M. Bergan, P. Gruffaz, Use of simple and complex in-vitro models for multiparameter characterization of human blood-material/device interactions, J. Biomat Sci. Polymer Edn, Vol 11, (2000).



Example methods – saline displacement blood draw



Example methods – vacuum blood draw



Example methods – saline displacement blood draw



Example methods – small blood volume = higher n

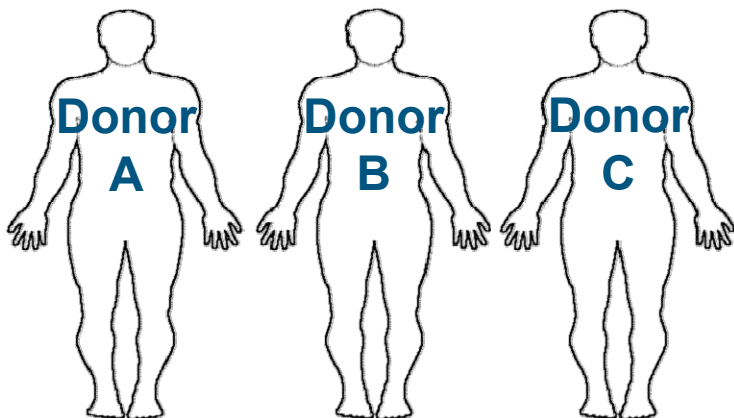


Example methods:

Stasis of
blood flow

Donor variability

Treat Donor as a variable!



Thrombin generation: phenotypic quantitation,
KE Brummel-Ziedens, RL Pouliot, KG Mann, J
of Thromb. and Hemost., 2, 281-288, 2003

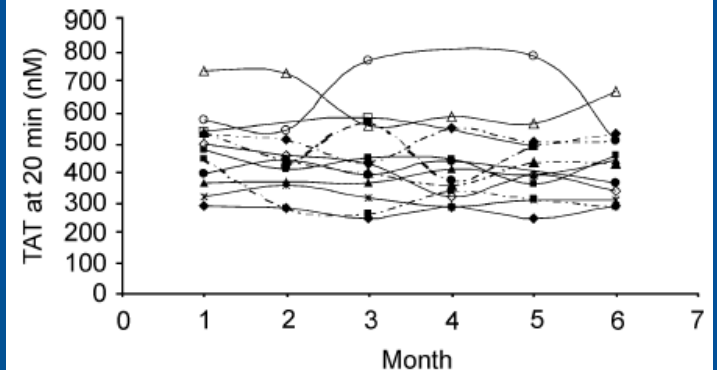
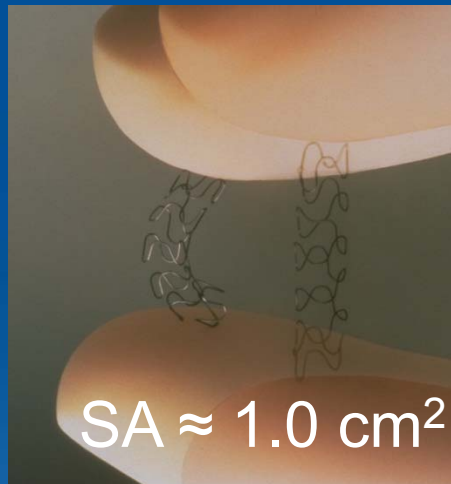


Fig. 2. Six-month individual thrombin profile. Thrombin generation as determined by thrombin-antithrombin III (TAT) at 20 min is illustrated for the 13 individuals over the course of a 6-month time period.

Example methods:



Stasis of
blood flow

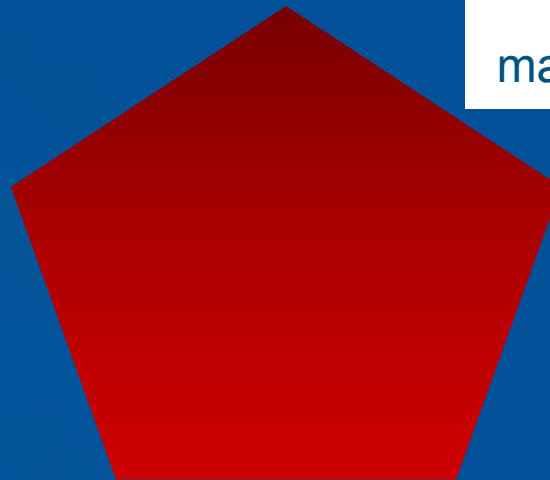
‘Exposure ratio’

$$= SA_M(\text{cm}^2)/\text{mL}_{\text{WB}}$$

=

Ratio of surface area of test
material to volume of blood (WB)

Donor variability



Medical device/
biomaterial

Biomaterial Surface



Example methods:

Stasis of
blood flow

Donor variability

Anticoagulants

Medical device/
biomaterial



Example methods:



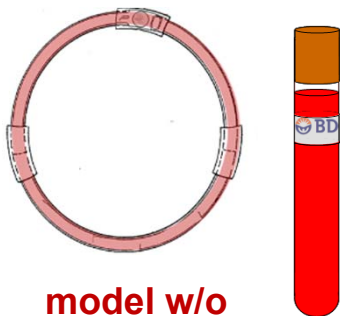
Stasis of
blood flow

Endothelial
injury

Donor variability

Anticoagulants

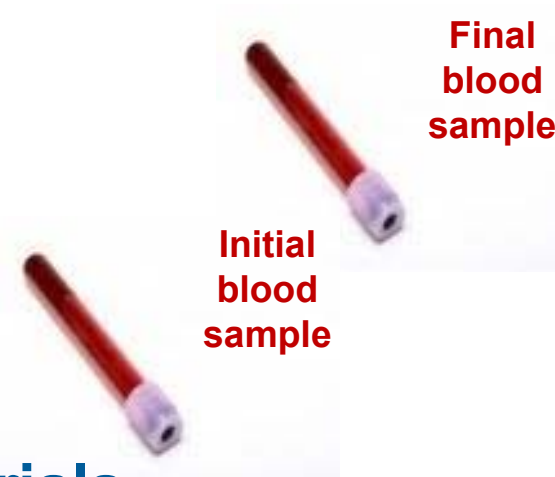
Medical device/
biomaterial



model w/o
materials

Materials

$$\text{'Exposure ratio'} = \frac{SA_M(\text{cm}^2)}{\text{mL}_{WB}} = 6.0 \text{ or } 9.0$$



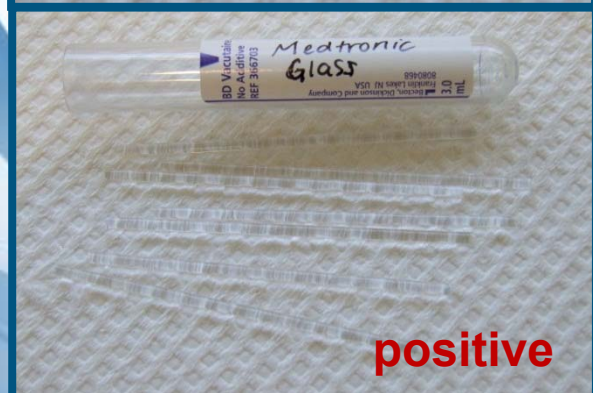
Initial
blood
sample

Final
blood
sample

Controls



negative



positive

Test Materials



Elasthane 80A
(Predicate material #1)



Pebax
(Predicate material #2)



heparin coated



heparin coated

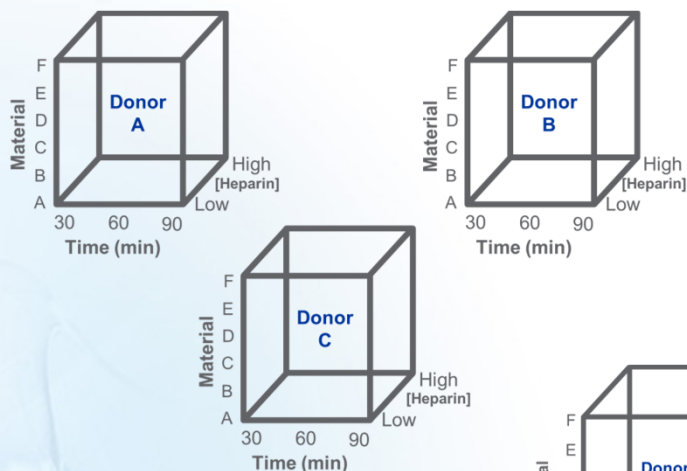


Example methods - Experimental designs

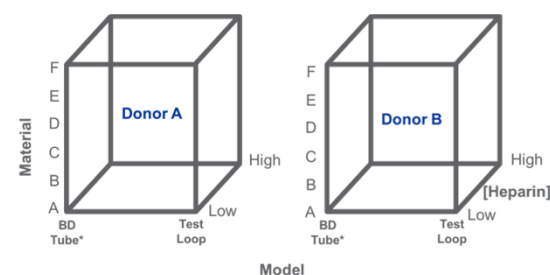
Table 1 DOE*	Model	*Ma- terial	Anticoagulant (U/mL blood)	Exposure Time (min) @ Temp	Exposure Ratio (cm ² /mL)	Donors	Blood Fill Method
DOE 1	Tube	A-F	Heparin @ 0.6 and 1.0	30, 60, 90 @ 37°C	9.0	3	saline displacement
DOE 2	Tube vs. Loop	A-F	Heparin @ 1.0 and 2.0	60 @37°C	9.0	2	saline displacement (loops) vacuum (tubes)
DOE 3	Tube vs. Loop	A-F	Heparin @ 1.0 and 2.0	60 @37°C	6.0	2	saline displacement

- A = PE (polyethylene), B = Glass, C = PEU (polyurethane), D = PEU+H (PEU with heparin coating), E = Pebax (polyether block amide), and F = Pebax+H (Pebax with heparin coating).
- * DOE = design of experiment

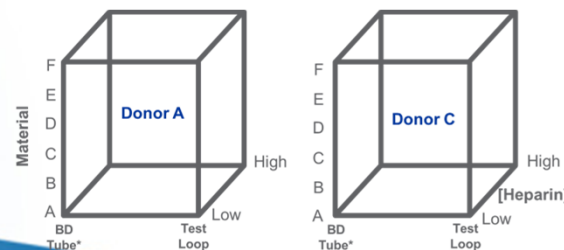
DOE 1



DOE 2



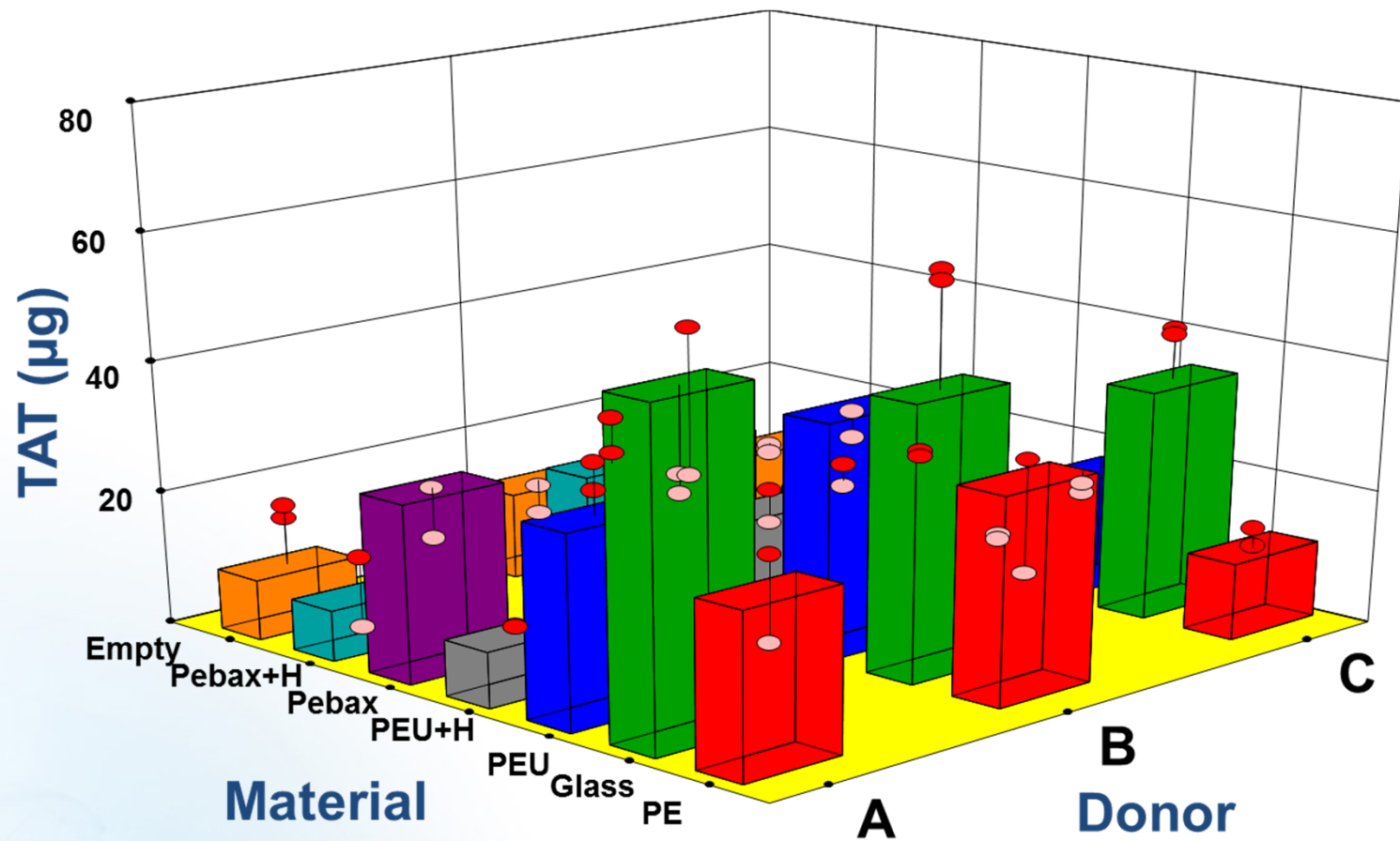
DOE 3



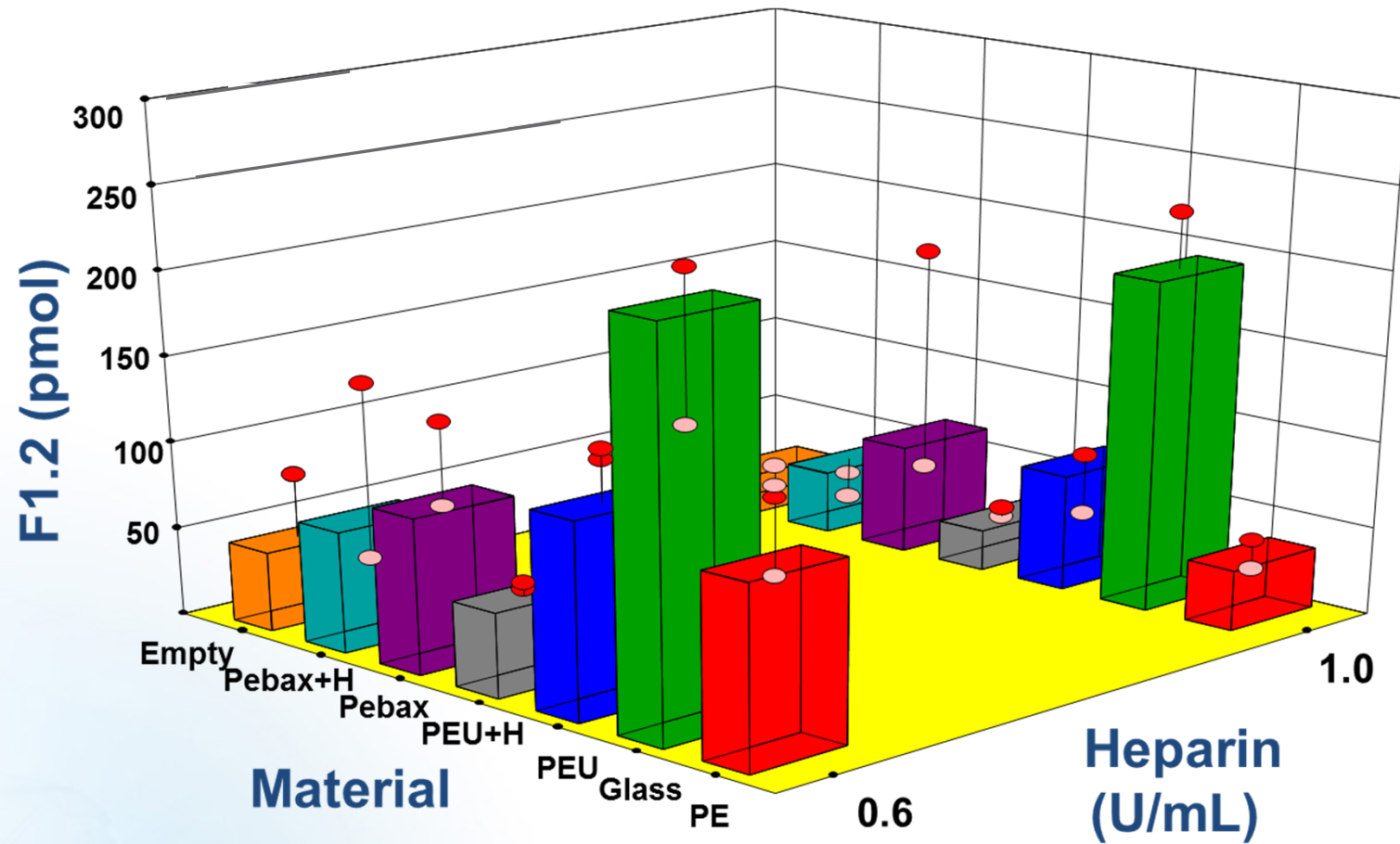
Example methods – ANOVA results

DOE	Protein	Model (A)	Material (B)	[Heparin] (U/mL) (C)	Donors (D)	Exposure Time (E)	Significant (P<0.05) Interactions
1	TAT	NA	P<0.0001	P<0.0001	P<0.0001	P<0.0001	BC, BD, BE, DE, CDE
2		NS	P<0.0001	P<0.0001	NS	NA	AB, AC, BC, CD
3		P<0.0001	P<0.0001	P<0.0001	NS	NA	None
1	F1.2	NA	P<0.0001	P<0.0001	P<0.0001	P<0.0001	BC, DE
2		NS	P<0.0001	P<0.0001	NS	NA	AB, AC, CD
3		P<0.0001	P<0.0001	P<0.0001	NS	NA	BD
1	βTG	NA	P<0.0001	P<0.0001	P<0.0001	NS	BD
2		P<0.0001	P<0.0001	P<0.0349	NS	NA	AB, CD
3		P<0.0001	P<0.0001	NS	NS	NA	None

Example methods – TAT graphical results

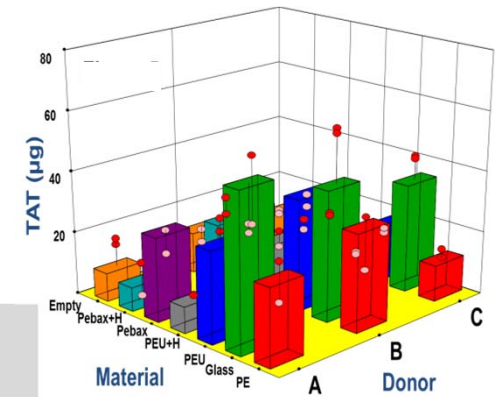


Example methods – F1.2 graphical results



Example methods – example rating scheme

Results	Score	Interpretation
$X < \text{empty}$	-1	Anti-thrombogenic
$X = \text{empty}$	0	Non-thrombogenic
$\text{empty} < X < \text{predicate}$	1	Low thrombogenicity
$X = \text{predicate}$	2	Predicate-consistent thrombogenicity
$\text{predicate} < X < \text{glass}$	3	Moderate thrombogenicity
$X \geq \text{glass}$	4	High thrombogenicity





Thank you

What might the format be of an *ideal standardized in vitro thrombogenicity test* for a test material?

